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Topical gel of Metformin solid lipid nanoparticles: A hopeful promise as a dermal delivery system

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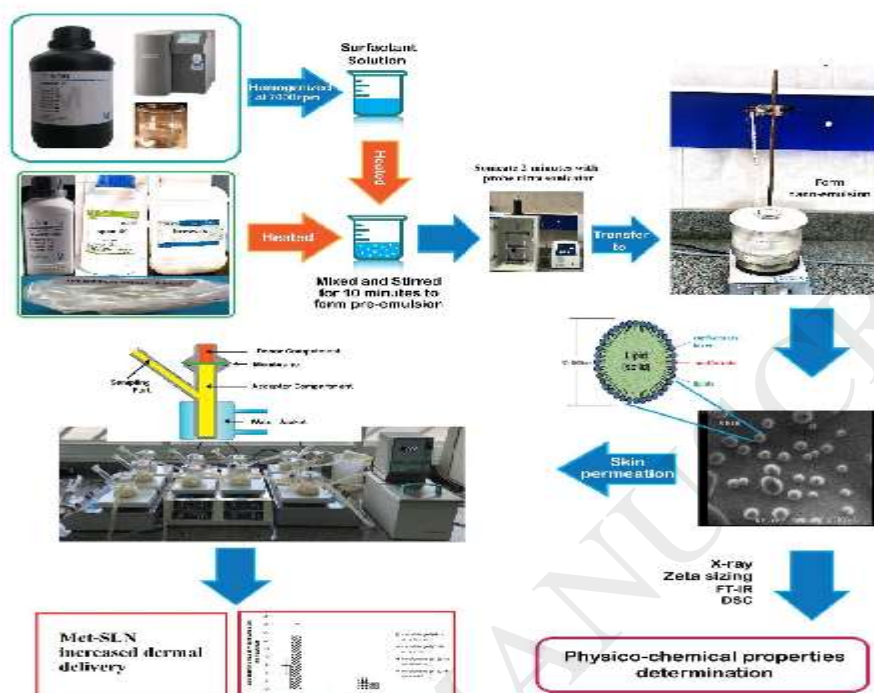
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Graphical abstract



Highlights

- Metformin solid lipid nanoparticles were prepared by the ultra-sonication method
- Solid state analysis ruled out any interaction between metformin and excipients
- Ratio of Tween:Span had a significant influence on performance of Met-SLN
- Gels containing Met-SLN showed better performance on the skin delivery of metformin

Abstract

The aim of the present study was to enhance the skin delivery of metformin by making solid lipid nanoparticles containing metformin using the ultra-sonication method. To achieve the optimum skin delivery for metformin, the effects of the ratio of two surfactants (Tween:Span) on nanoparticles properties and their performance were investigated. Photon correlation spectroscopy, scanning electron microscopy (SEM), Powder X-ray Diffractometer (PXRD), Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC) were used to characterize the solid state of metformin in solid lipid nanoparticles. Generally, the particle size of nanoparticles decreased by the addition of co-emulsifier (Span®60). Results showed that all formulations made by binary mixtures of surfactants had low particle size, low Polydispersity index and high zeta potential. It was interesting to note that the smallest nanoparticles (203.8 ± 15.356) was obtained when the HLB of the binary surfactants (HLB of 11.67) was closer to the HLB of beeswax (HLB of 12) used in the preparation of SLN. It was also found that by decreasing the HLB of the system from 14.9 to 10.06 the zeta potential of SLNs increased from -0.651 ± 0.315 to -6.18 ± 0.438 mV. But, a further reduction in the HLB from 10.06 to 8.45 caused a reduction in the zeta potential from -6.18 to -3.596 ± 0.255 . Results showed that the highest entrapment efficiency of $45.98 \pm 9.20\%$ was obtained for formulation with larger particle size and with the highest HLB value (HLB 14.9). DSC study showed that metformin in SLN is in an amorphous form. FT-IR spectra of Met-SLN showed that the prominent functional groups existed in the formulations which could be an indication of good entrapment of metformin in a lipid matrix. FT-IR results also ruled out any chemical interaction between the drug and the excipients. The amounts of metformin detected in the skin layers and the receptor chamber at all sampling times were higher for nanogel compared to metformin gel.

This is an indication of a better performance of Metformin nanogel *ex-vivo* and could be developed further for clinical studies.

Keywords: Metformin, Solid lipid nanoparticles, Ultra-sonication, DSC, FT-IR, Skin permeation

1. Introduction

In recent years the anti-inflammatory effect of metformin (Met), which is widely used as an anti-diabetic drug, has been reported in many animal models. Met inhibits the production of reactive oxygen species (ROS) from nicotinamide adenine dinucleotide hydride (NADH) in lipopolysaccharide-activated macrophage [1-3]. Inflammation and the accumulation of ROS perform a vital role in the intrinsic and photoaging of human skin [4]. One of the major causes of skin ageing is chronic exposure to ultraviolet (UV) radiation. UVB (ultraviolet B-rays) is the light from the sun that has a fairly short wavelength and penetrates the skin and activates three major skin ageing cascades by inducing ROS production which is responsible for UVB-induced skin ageing [5]. In order to get a benefit from the anti-inflammatory effect of Met on the skin, the best option is to enhance its dermal effects, therefore, its side effects will be less through the topical administration route [6]. Met hydrochloride belongs to class I drugs (based on BCS classification) and its permeability is the rate-limiting factor that could be modified by altering the physiochemical properties of the drug which is a very complex task. Nanotechnology is another approach which can improve percutaneous drug absorption effectively without any chemical change in the molecular structure of drugs [7]. Among all colloidal carriers, Solid Lipid Nanoparticles (SLNs), due to their advantages such as improved physical stability, good tolerability, and ease of scale-up and manufacturing, have emerged as an alternative colloidal carrier [6]. Recently, SLNs have been widely used for skin delivery due to their safe interaction with skin layers, and improved skin permeation [6]. However, encapsulation of hydrophilic

drugs into the hydrophobic lipid of SLNs is a major problem because during the production process the drug tends to partition towards the aqueous phase. To best of our knowledge, there are very limited examples of hydrophilic drugs being encapsulated into SLNs [8-11], these include the loading isoniazid [8], ciprofloxacin [9, 10,] and gentamicin sulfate [11] on SLN. In these research work, different techniques such as microemulsion [9], ultrasonication and high-speed homogenization [10-11] were employed to manufacture SLN. None of the above studies investigated the effect of HLB value of binary surfactants on the manufacturing of solid lipid nanoparticles containing an active pharmaceutical ingredient. Met was selected as a hydrophilic and anti-ageing drug to incorporate in SLNs by the ultra-sonication method, and the effect of HLB of surfactant mixtures on the properties of SLN was explored. The optimized Met-SLN was formulated as a topical gel and applied on the skin for further investigation. Based on the literature survey, the investigation on the effect of a mixture of surfactants on properties of hydrophilic drugs incorporated in solid lipid nanoparticles is lacking.

2. Materials and Methods

2.1 Materials

Met was obtained from Tehran Chemie pharmaceutical Co. (Tehran, Iran). Tween 60 and cholesterol were obtained from Merck (Merck Co., Germany). Span 60 was obtained from Daejung (Daejung Chemicals & Metals Co., Ltd. Korea). Beeswax was obtained from John's Laboratory (John's laboratory chemicals, India). Distilled water was purified using a Human power 2 system (human Co., Korea).

2.2 Preparation of Met-SLN

Met nanoparticles were prepared by the probe ultra-sonication method [8]. The mixture of beeswax, cholesterol, span 60 and Met were melted at below 70 °C by a heater stirrer. The surfactant solution (Tween 60 and water) was heated to 75-80 °C. The pre-heated surfactant solution was mixed with the heated mixture of lipids and Met to form a pre-emulsion using a hotplate magnetic stirrer. The mixture was sonicated for 2 minutes using a probe sonicator (Bandelin, 3100, Germany) and immediately immersed in an ice bath and stirred at 300 rpm after the sonication process finished. For more details on the composition refer to Table 1.

2.3 Preparation of Met-SLN gel and conventional metformin gel

To prepare the plain gel, carbopol (8%) was dispersed in water and kept overnight. Then the carbopol solution was neutralized by triethanolamine. To prepare met-SLN gel, 26 g of Metformin nanodispersion (equal to 0.179% metformin) was mixed with 5 g plain gel under propeller homogenizer at 400 rpm. To prepare conventional metformin gel, metformin solution (equal to 0.179% metformin) was mixed with 5 g plain gel under propeller homogenizer at 400 rpm.

2.4 Physicochemical characterization

The average particle size, polydispersity index (PDI) and zeta potential of Met-SLN were determined by dynamic light scattering (DLS) method using a Zetasizer Nano ZS system (Malvern Instruments, Worcestershire, UK) at 25°C with an angle of 90° [6].

2.5 Entrapment efficiency and Drug loading

The Met-SLNs were subjected to centrifugation for 120 minutes at 27000 rpm (Sigma, Germany) to separate loaded Met from the dispersion. The supernatant was filtered using a syringe filter (pore size: 0.22 μm) and the amount of Met in the supernatant (free drug) was determined by HPLC Kneuer which was equipped with the Kneuer XDB-C18 column (5 μm , 4.6 \times 250 mm). The mobile phase was a 60: 40 (v/v) phosphate buffer (1 mM) and Acetonitrile. The flow rate was 1ml/min and UV detection was carried out at 225 nm. The retention time of the drug was 4 minutes. Drug entrapment efficiency (EE %) and drug loading were calculated by equations 1 and 2 respectively:

$$EE \% = \frac{W_{\text{initial drug}} - W_{\text{free drug}}}{W_{\text{initial drug}}} \times 100 \quad (\text{Equation 1})$$

$$DL\% = \left(\frac{W_{\text{initial drug}} - W_{\text{free}}}{W_{\text{Lipid}}} \right) \times 100 \quad (\text{Equation 2})$$

Where $W_{\text{initial drug}}$ is the amount of drug added in the formulation, $W_{\text{free drug}}$ is the amount of drug in supernatant and W_{lipid} is the amount of lipid [12].

2.6 Scanning Electron Microscopy (SEM):

The SEM analysis was performed for morphological studies. The formulation coated with gold in nanostructure coating DSR1, and observed in HITACHI S-4160 SEM at an acceleration voltage of 20.0 kV and a magnification of 15000 X [13].

2.7 Fourier Transforms Infrared (FT-IR) analysis

For FT-IR studies, SLN were separated from SLN suspension by centrifugation using a 3-30ks sigma centrifuge. The separated SLNs was dried under reduced pressure using an alpha 1-2 ld plus freeze dryer (Marin Christ, Osterode, Germany). The dried SLNs, Met, Beeswax, cholesterol were used for FT-IR studies using a Perkin Elmer FT-IR spectrophotometer (Perkin FTIR-One, USA) from 400 to 4000 cm^{-1} to evaluate any alteration in the molecular levels. The sample was grounded with KBr and compressed into a suitable-size disk (13 mm) for measurement [6].

2.8 Differential Scanning Calorimetry (DSC)

DSC measurements were performed on the bulk ingredients and Met-SLN powder using a Pyris 6, PerkinElmer, USA). The samples (5 mg) of bulk lipid, drug and freeze-dried SLN formulations were weighed in aluminium pans and sealed. The sealed pans were kept under isothermal conditions at 20 $^{\circ}\text{C}$ for 30 minutes. After equilibration, DSC thermograms for the bulk samples and the Met-SLN powder were recorded from 20 $^{\circ}\text{C}$ to 250 $^{\circ}\text{C}$ at a heating rate of 20 $^{\circ}\text{C}/\text{min}$, under inert nitrogen gas atmosphere [6]. The DSC was calibrated using indium before testing the samples.

2.9 Powder X-ray Diffractometer (PXRD) analysis

The diffraction patterns were obtained using a D8 Advance X-ray diffractometer (Bruker-binary V2, Germany) (40 kV, 30 mA) to identify any changes in the crystal lattice of the materials after making SLNs. The crystalline characteristics of Met, beeswax, cholesterol and freeze-dried SLN

powder were analyzed by exposing them to Cu K α radiation with a wavelength of 1.5406Å and scanned from 5.000° to 70.000°, 2 θ at a step size of 0.040° and step time of 1s [6].

2.10 *In vitro* skin permeation study

The study was compatible with the Ethical Guidelines for Investigations in Laboratory Animals and was approved by the Ethics Review Committee for Animal Experimentation of Mazandaran University of medical sciences. The abdominal skins of male Wistar rats (weighing 120-150 g) were anaesthetized (given 87 mg ketamine/kg of body weight and 13 mg xylazine/kg) and shaved using electric hand razors. After 48 h the rats were killed by chloroform inhalation and the abdominal skin was surgically removed. The skin was carefully cleaned from adhering subcutaneous fat and put in contact with a saline solution for 24 h before initiating the diffusion experiment. The skin was set in Franz cells (with a diffusional area of 3.8 cm²). The excised skin was placed between the cell halves and the dermis faced the receptor fluid. The receiver compartment was filled with deionised water. The diffusion cells were maintained at 32±0.5 °C (using thermostatically controlled water which was circulated through a jacket surrounding the cell body throughout the experiments) and stirred at 150 rpm with magnetic stirring bars throughout the experiment. The receptor temperature is usually set to 32 degrees °C to approximate normal skin conditions. 0.4 g (equal to 600 µg metformin) of Met-SLN gel (as a sample) and 0.4 g (equal to 600 µg metformin) of conventional metformin gel (as control) were spread out uniformly on the shaved dorsal surface in the donor compartment which was sealed from the atmosphere by gentle rubbing using a spatula. Samples were withdrawn from receiver medium at predetermined time intervals (4, 6, 8, 10 and 24 h) and an equivalent volume of fresh deionised water was added to the receiver phase. All samples were filtered by a syringe filter

(pore size: 0.22 μm) and analyzed by the HPLC method as described before (section 2.4). The skin was removed at the end of the permeation study and washed three times with deionised water and dried to calculate the amount of Met deposited within the skin. The removed skin was cut into small pieces using a pair of scissors and transferred to a tube and digested 24 h in water and then sonicated for 1 h with bath sonicator. The supernatant filtered through a filter paper and then filtered by a syringe filter (pore size: 0.22 μm) and quantified by HPLC for Met content [6].

2.11 Statistical analysis

Results are shown as the mean \pm standard deviation of at least three determinations ($n = 3$). The treated groups were compared to the control by analysis of variance (ANOVA) followed by the Tukey's test. In the case of comparison of only two means, a t-test was performed. The statistical analysis was carried out using the SPSS 2021 software. A P-value < 0.05 was considered as significant.

3. Results and discussion

3.1 Nanoparticle characteristics (size, PDI, zeta potential and entrapment efficiency) analysis

As the particle size of nanoparticles is an important characteristic of nanoparticles, therefore, a special attention was made on this factor. Table 1 shows the hydrodynamic diameter (intensity weighted mean diameter or the z-average diameter) of nanoparticles and the PDI reflects the quality of the dispersion (as an indication of the width of the particle size distribution) [14]. The PDI value usually ranges from 0 to 1 and the PDI values > 0.7 indicates a very broad distribution of particle sizes [15]. Table 1 shows that the size of nanoparticles and PDI value varied by the changes in the composition of surfactants. The use of binary mixtures of surfactants with low and

high HLB values generally ensures a better stability of emulsion droplets. The surfactants with high and low HLB can be dispersed in the aqueous and the oily phase respectively leading to a more stability of the surfactant film at the interface [6]. Results showed that all formulations containing binary mixtures of surfactants have low particle size and Polydispersity index (compare F1 with F2-5 in Table 1 and Fig. 1). This clearly showed that the addition of co-emulsifier (Span[®]60) reduced the particle size which could be an indication of a better stability of the dispersed product in the presence of the second emulsifier [14]. In other words, it was interesting to note that by adjusting the HLB value to 11.67 (near beeswax HLB) the particle size of SLNs significantly decreased from 790.733 ± 117.75 nm to 203.8 ± 15.356 nm ($P < 0.05$) (compare F3 with F1 in Table 1 and Fig. 1). The prepared SLNs dispersions by a binary mixture of surfactants had a PDI value ≤ 0.6 indicating an acceptable distribution of SLNs. Results showed that, although, the drug particles have positive charge, the zeta potential of the different formulations was negative and was in the range of -0.651 mV to -6.180 mV. It has been reported that the nonionic surfactants surprisingly exhibit a negative zeta potential around colloidal particles which could be due to the dipole nature of the ethoxy groups of nonionic surfactants [16]. The table also shows that by decreasing the ratio of Tween: Span from 1:0 to 1.11:1 the absolute zeta potential of SLNs increased from -0.651 ± 0.315 mV (F1) to -6.18 ± 0.438 mV (F4) ($P < 0.05$) and particles became more negatively charged. It seems that, the tendency of binary mixtures of Tween and Span increases compared to when single surfactant was used and binary surfactants can form more condensed barrier around colloidal particles and make particles more negatively charged as a result of more surfactants around nanoparticles (it was already discussed that nonionic surfactants produce negative charge around colloidal particles). But, this was not the case for formulation F5 where the amount of Tween becomes less than the amount of Span in

the formulation. This indicates that the ratio of Span to Tween has a significant effect on zeta potential and the highest zeta potential was obtained when the ratio of these two surfactants is close to each other. This is interesting results as the ratio of these two surfactants not only controls the zeta potential but also the size of nanoparticles (Table 1). Generally, smaller nanoparticles carries more surfactants around it and making nanoparticles more negatively charged. This was not the case when formulation F4 was compared to formulation F3 as apart from the size, it seems the amount of surfactants adsorbed by the nanoparticles can change the zeta potential.

Somehow, the presence of high concentration of Span defects the film layer formed by Tween causing a reduction in the zeta potential (compare zeta potential of F4 and F5 in Table 1) as a result of less surfactants around nanoparticles. The electrophoretic mobility of the particles reduced by the surface coverage of SLNs and lowers the zeta potential [14]. As the critical micelle concentration of Tween 60 is relatively low (about 0.022 mM), therefore, the concentration of Tween 60 monomers in the dispersion medium should be low [17]. This fact indicates that adsorption of surfactant monomers onto the hydrophobic surfaces of the lipids is more likely to happen rather than forming micelles. At the interface between two immiscible liquids such as oil and water, surfactants will orientate themselves with their hydrophilic group in the water and their hydrophobic group in the oil [14]. Also, a further reduction in the ratio of Tween:Span to 0.583:1 in F5 caused a reduction in the zeta potential of SLNs from -6.18 ± 0.438 mV to -3.596 ± 0.255 mV. The surface of particles perfectly is covered by nonionic surfactants and use of the high concentration of second surfactant (in this case Span) probably can break fully the coverage ability of the first surfactant [14]. Based on the effect of the ratio of binary surfactants, it can be concluded that the use of the second surfactant probably at high

concentration can break the surfactant film around the particles and reduces the zeta potential. In the current study, the zeta potential value was not considered a primary parameter in the selection of the optimal formulation. SLNs perfectly covered by nonionic surfactants like Tween and Span 60 tend to remain stable because of greater steric stabilization despite having a lower zeta potential and less electrostatic stabilization [14]. Results of the EE% measurements showed that the EE% of the different formulations were in the range $45.986 \pm 9.209\%$ to $28.503 \pm 5.610\%$. The loading quantity could be affected by the drug solubility and miscibility in the lipid matrix, and the lipid phase polymorphic state. To decrease the degree of lipid phase crystallinity and to increase loading capacity, cholesterol was added to the lipid phase [18]. Metformin has a high tendency to escape from lipid matrix due to its high solubility in water [19]. As particle size decreases the specific surface area increases and thus drug loading decreases. Ebrahimi *et al.* showed that the loading values increased as the particle sizes increased [20]. The reverse relationship between particle size and loading parameters (loading efficiency and entrapment efficiency) has been shown in previous studies. The higher surface area increases the mobility and tendency of the drug to escape from the matrix. As in smaller particles, the total particle surface is larger and drug repulsion is higher, therefore, this resulted in lower EE% [20]. The highest EE% belonged to formulations with highest HLB value= 14.9 with larger particle size. Xu *et al* prepared SLNs containing Met for cellular and mitochondrial uptake. The mean particle size, zeta potential, entrapment efficiency, and loading efficiency of Met-SLNs were 102.3 ± 4.16 nm, -21.25 ± 4.89 mv, and $26.25 \pm 2.59\%$ respectively [12]. In another study, Ngwuluka *et al* prepared Met-loaded solid lipid nanoparticles (SLN) for Colon Cancer by double emulsification-solvent evaporation employing cold homogenization. The particle size, zeta potential and percentage drug entrapment for their optimized SLN were 195.01 ± 6.03 nm, -17.08 ± 0.95 mV

and $29.30 \pm 2.29\%$ respectively [21]. In another study, Sharma *et al* prepared Met-loaded solid lipid nanoparticles for transdermal delivery by solvent diffusion technique. The particle size, zeta potential and percentage drug entrapment for their optimized SLN were 242 ± 5 , $+27$ mV and $94.62 \pm 0.02\%$ respectively [22]. The advantage of the current method used to prepare Met-SLN over other published method is that no organic solvent was used, and the nanoparticles were incorporated into the gel base which could be a finished product. In addition, none of the above studies investigated the effect of Span:Tween ratio which is needed to optimize Met-SLN to have a better performance. The optimized Met-SLNs (F3) prepared by ultra-sonication method had the particle size of 203.8 ± 15.356 nm, PDI of 0.583 ± 0.0085 , the zeta potential of -4.723 ± 0.321 mV and the percentage drug entrapment of $32.92 \pm 1\%$. It was interesting to note that the concentration of metformin used in the current study was 7.5 times higher than the concentration of the drug used in another study [9] and the loading of drug in the current study was over 2 times higher than Met-SLN reported by Ngwuluka *et al* [18]. Gandomi *et al* loaded Ciprofloxacin HCl, a water-soluble drug, by a microemulsion technique in SLNs. Comparing their results with the current study showed that the type of drug can change the EE and DL values. For example, they produced a lower mean particle size for SLNs (95 ± 3 nm) and lower their zeta potential (-1.9 mv) compared to the current study [9]. Shah *et al* loaded ciprofloxacin HCl in SLNs and they showed that changing the amount of lipid, stirring speed and stirring time could remarkably modify the entrapment efficiencies and particle size of the prepared SLNs. The particle size of an SLN was in the range of 159 nm to 246 nm and The highest entrapment efficacy (EE) was reported to be 39.5% [10]. In another study, Kazemi *et al* loaded gentamicin sulfate, a water-soluble antibiotic, by an ultrasonication/high speed homogenization technique on SLNs and they showed that the average particle size of gentamicin-loaded SLNs was 282.3 nm

and its zeta potential was +8.16 mV with drug-loading efficiency of 40%. Although, all the drugs used in the mentioned studies were water-soluble, but the solubility of isoniazid was extremely higher than the drugs used in other studies. The water solubility of isoniazid, ciprofloxacin HCl and gentamicin at 25°C is 125, 35 and 50.0 mg/mL respectively but metformin HCl with a solubility of 1060 mg/mL at 25°C was much higher compared to the other hydrophilic drugs used.

3.2 SEM analysis

Particle size and morphology of nanoparticles were also studied by scanning electron microscopy (SEM) (HITACHI S-4160). The image obtained for F3 by SEM (Fig.2) showed the typical morphological aspects of nanoparticles. The SEM image showed that the particles were segregated, uniform in size and spherical in shape.

3.3 FT-IR

Figure 3 demonstrates the infrared spectra of Met, cholesterol, beeswax and Met-SLN (formulation 3). The diagnostics peaks of Met were found in the FT-IR spectra of Met-SLN formulation. Cyanides group's peaks of Met were still recognizable, which could be an indication of a good entrapment of Met in a lipid matrix. On the basis of FT-IR results shown, the results proved that there is no chemical interaction between the drug and its excipients as main diagnostic peaks for the drug are apparent in Figure 3. The results of FT-IR spectra of pure Met, cholesterol and beeswax are summarized as follows:

Met: 3371 cm^{-1} (first N-H, stretching), 3293 cm^{-1} (second N-H, stretching), 3174 cm^{-1} (C-H, stretching), 1623 cm^{-1} (C=N, stretching), 1446 cm^{-1} (C-H, bending), 1059 cm^{-1} (C-N, stretching). Cholesterol: 3400 cm^{-1} (OH, stretching), 3035 cm^{-1} (cyclic C-H, stretching), 3000-2850 cm^{-1} (methyl C-H, stretching), 1445 cm^{-1} (C=C, stretching), 1220 cm^{-1} (C-O stretching). Beeswax: 3000 cm^{-1} (OH, stretching), 2950-2850 cm^{-1} (C-H, stretching), 1737 cm^{-1} (C=O, stretching), 1470-1376 cm^{-1} (C-H, bending), 730 cm^{-1} (long chain hydrocarbon and steric group, Vibration).

3.4 Differential Scanning Calorimetry (DSC)

The thermal behavior of metformin, beeswax, cholesterol and Met-SLN powder (F3) were investigated by DSC and their DSC thermograms are shown in Figure 4. Met and cholesterol thermograms showed a single sharp endothermic peak at about 235 °C and 150 °C respectively corresponding to their melting points, an indication of highly crystalline nature for these two materials. Beeswax showed an endothermic peak around 40-66 °C corresponding to its melting point. The DSC thermograms of investigated SLN formulation only contained the endothermic peak around beeswax melting point, but the endothermic peak of drug disappeared (Fig.4) which suggests that the metformin in SLN is in an amorphous state or is molecularly dispersed within the SLN.

3.5 Powder X-ray Diffractometer (PXRD) analysis

Figure 5 illustrates the X-ray diffraction (PXRD) pattern of Met, cholesterol, beeswax and Met-SLN (F3). The PXRD of Met revealed the distinct peaks at 2θ : 12.92°, 12.596°, 17.463°, 22.662°, 23.119°, 24.373°, 25.254°, 26.282°, 27.058°, 29.272°, 31.189°, 32.57°, 34.22°, 37.024°, 37.52°, 39.2°, 40.6°, 42°, 44.58°, 46°, 47.3°, 48.7°, 49.63° [23]. The cholesterol showed the peaks

at 2θ : 5.272° , 10.604° , 12.83° , 15.501° , 16.972° , 17.393° , 18.154° , 23.565° , 26.244° , 37.151° , 42.404° . These sharp peaks for both of Met and cholesterol confirmed the highly crystalline nature of them. The beeswax showed the peaks at 2θ : 8.698° , 19.553° , 21.656° , 24.025° , 30.12° , 36.252° , 40.45° , 47.33° , 55.13° indicating the crystalline nature of the beeswax. The PXRD pattern of Met-SLN showed the peaks at 2θ : 21.384° , 23.717° , 40.23° , 42.97° and 48.34° . As observed in Fig.5, lipid peaks did not shift but had a reduced intensity as compared to free lipid. This may be attributed to the incorporation of cholesterol and Met between the parts of the beeswax leading to a change in the crystallinity of the lipid. The results showed a good entrapment of Met in beeswax without any interaction which are aligned with FT-IR and DSC analysis.

3.6 *In vitro* percutaneous absorption study

To assess the performance of both Met-SLN nanogel and compare it with the performance of the standard Met gel (just containing 0.15% Met in carbopol gel) *in vitro* percutaneous absorption was performed. In this test, the ability of Met-SLN to penetrate different layers of skin and its skin permeation were considered and the results are shown in Figures 6 and 7. Nanogel showed an increased penetration into and through the skin layers. This indicates that the novel Met-SLN gel formulation can have a better local effect compared to Met gel. Results suggest that the more anti-ageing effect could be expected for drug-loaded SLNs gel compared to plain drug gel. Madan *et al* also showed that skin deposition of the mometasone from gels containing SLN was 2.67 times more than marketed cream and 20 times more than plain drug loaded gel [24]. Bhalekar *et al* also showed that the miconazole nitrate -SLN gel could significantly increase the accumulative uptake of miconazole nitrate in the skin over the marketed gel and showed a

significantly enhanced skin targeting effect [25]. Sharma *et al* also showed that the high cumulative amount of Met is permeated from Met SLN transdermal patches [22]. The results obtained from *in vitro* percutaneous absorption in the present study indicated that lipid nanoparticles gel are promising in local delivery for Met and other hydrophilic drugs and stimulating new and deeper investigations in the field.

4. Conclusion

The present study suggests that solid lipid nanoparticles are suitable carriers for skin delivery of Met and other hydrophilic drugs. This carrier can increase the dermal delivery of metformin with a higher concentration into deeper layers of the skin. In order to achieve more stable nanoparticles and localize the delivery of metformin, the solid lipid nanoparticle formulations need optimization and the research showed that Span: Tween ratio was a critical parameter to optimize metformin nanogel formulation to achieve a good delivery to the skin.

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Table 1. Component and physicochemical properties of investigated Met- SLN (% w/w). The data are the mean and standard deviation of three determinations (n = 3). Metformin: Met; PDI: polydispersity index; zeta potential; EE: entrapment efficiency.

Formulation	Met (%)	Cholesterol (%)	Beeswax (%)	Span 60 (%)	Tween 60 (%)	Water (%)	Size (nm)	PDI	Zeta potential (mV)	EE (%)	HLB
F1	0.179	0.179	3.22	0	6.81	89.60	790±117	1±0	-0.651±0.31	45.98±9.20	14.9
F2	0.179	0.179	3.22	1.07	5.73	89.60	258±6	0.545±0.01	-2.11±0.13	28.50±5.61	13.28
F3	0.179	0.179	3.22	2.15	4.65	89.60	203±15	0.583±0.00	-4.723±0.32	32.92±1.75	11.67
F4	0.179	0.179	3.22	3.22	3.58	89.60	263±8	0.581±0.01	-6.18±0.44	37.32±0.93	10.06
F5	0.179	0.179	3.22	4.30	2.50	89.60	389±50	0.537±0.00	-3.596±0.26	29.38±3.55	8.45

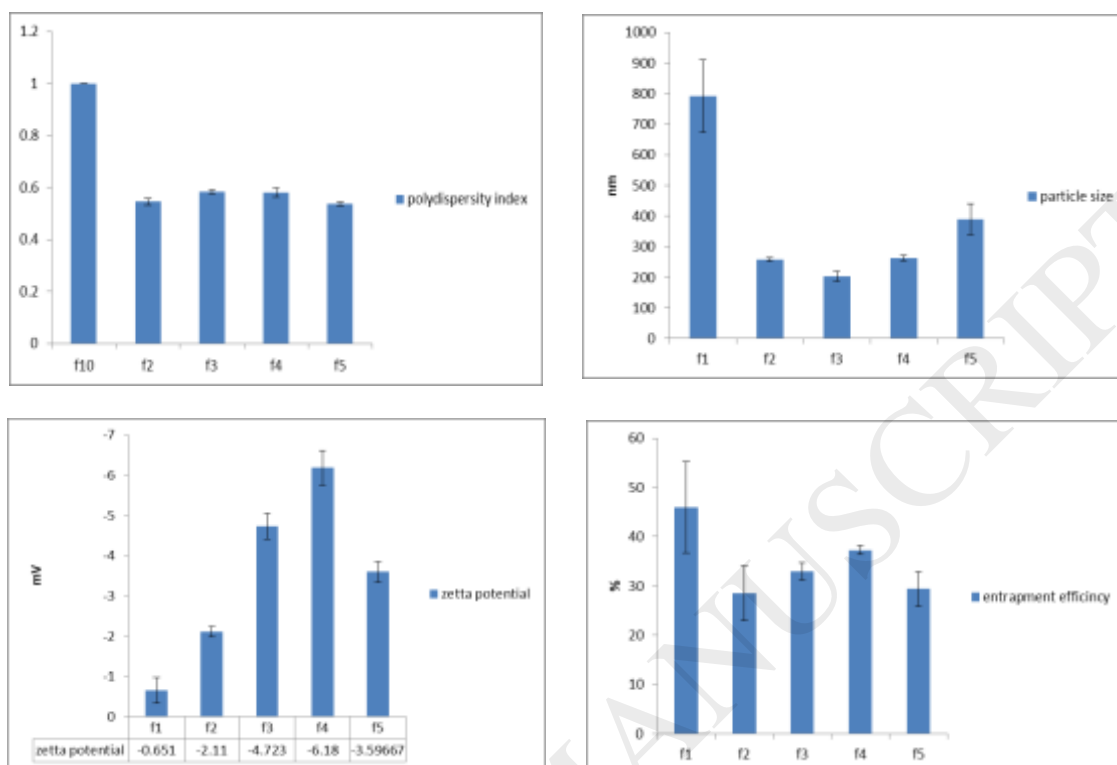


Figure 1. Particle size, polydispersity index, zeta potential and entrapment efficiency for various formulations (error bars are standard deviation, ANOVA test followed by Tukey's test was carried out between the formulations. The difference was significant $p < 0.05$; $n = 3$).

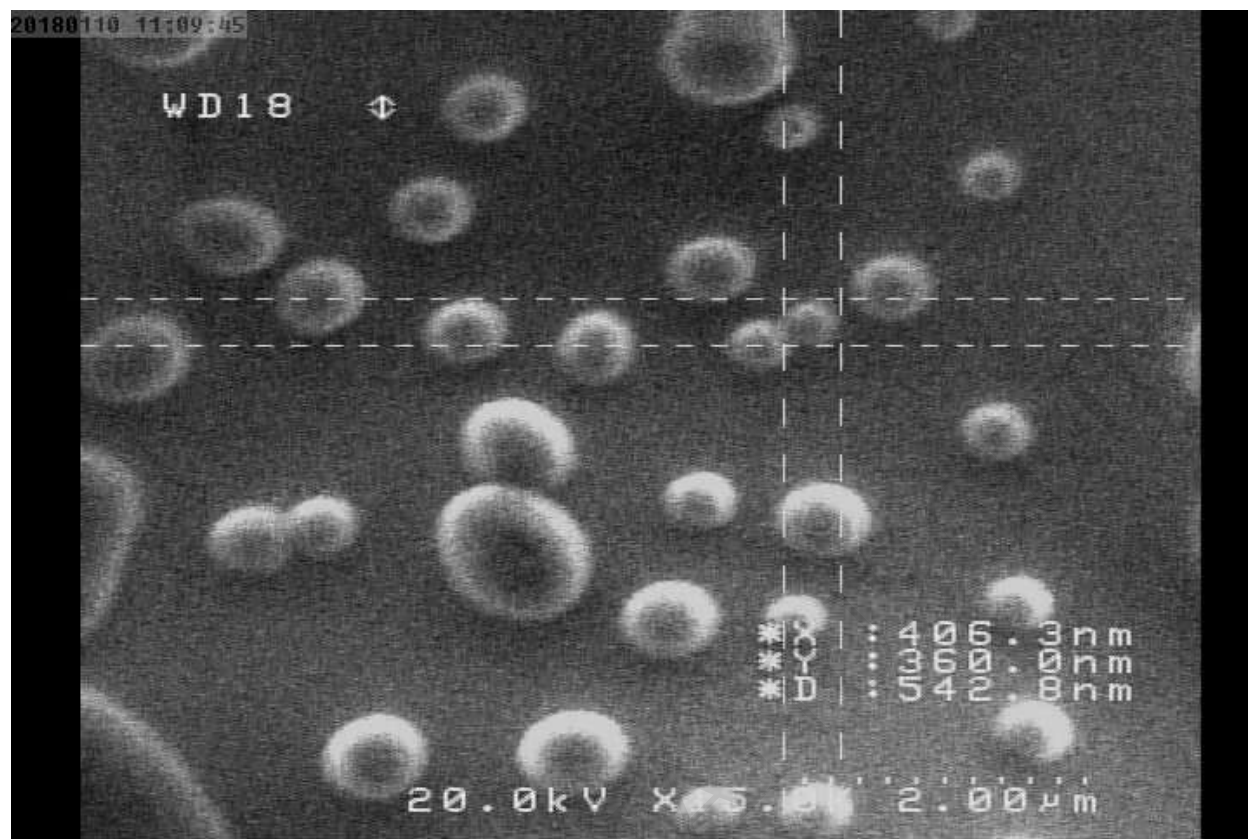


Figure 2. SEM micrographs of Met-solid lipid nanoparticles (Met-SLN3).

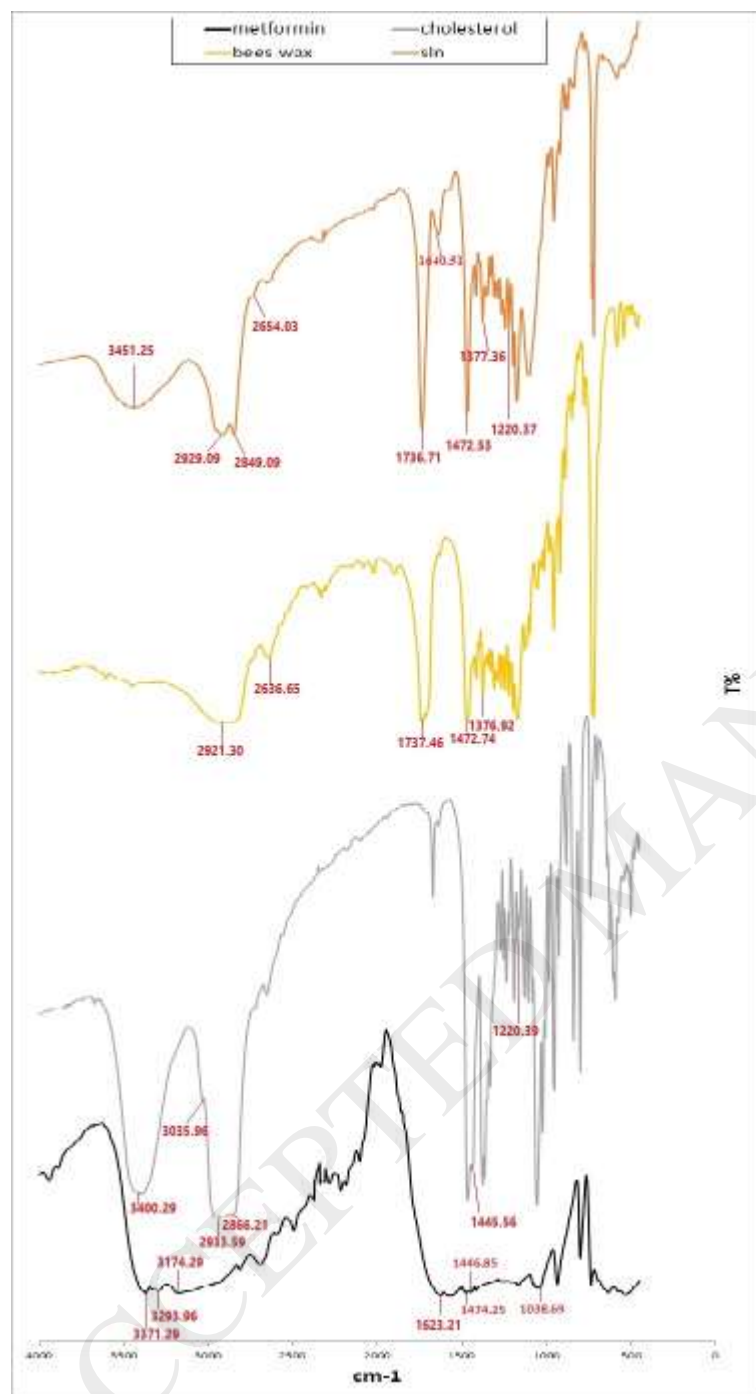


Figure 3. FT-IR spectra of Met, beeswax, cholesterol and Met-SLN

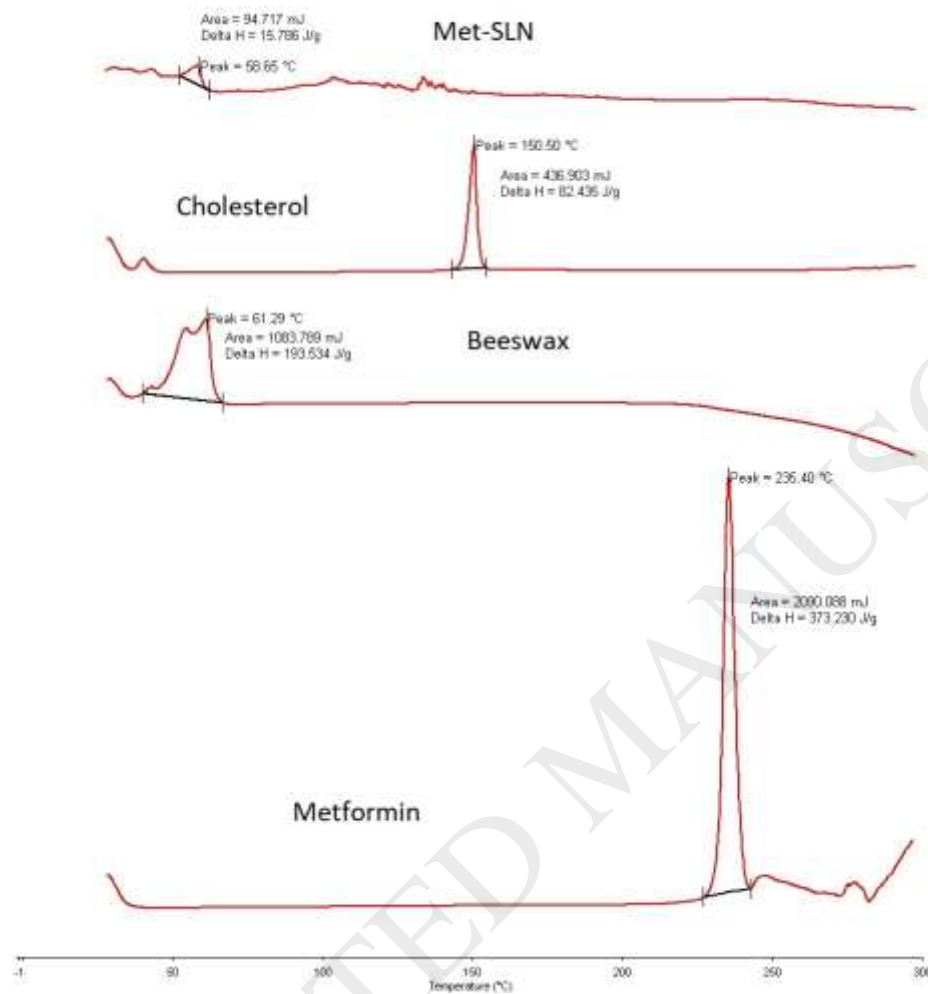


Figure 4. DSC traces of Met, beeswax, cholesterol and Met-SLN

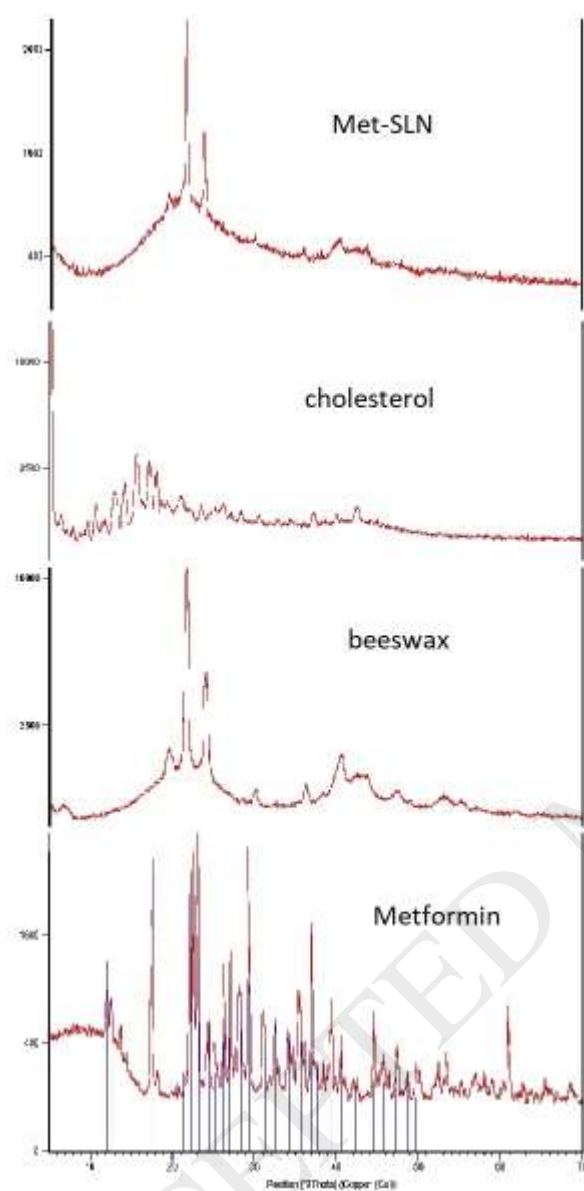


Figure 5. XRD of Met, beeswax, cholesterol and Met-SLN

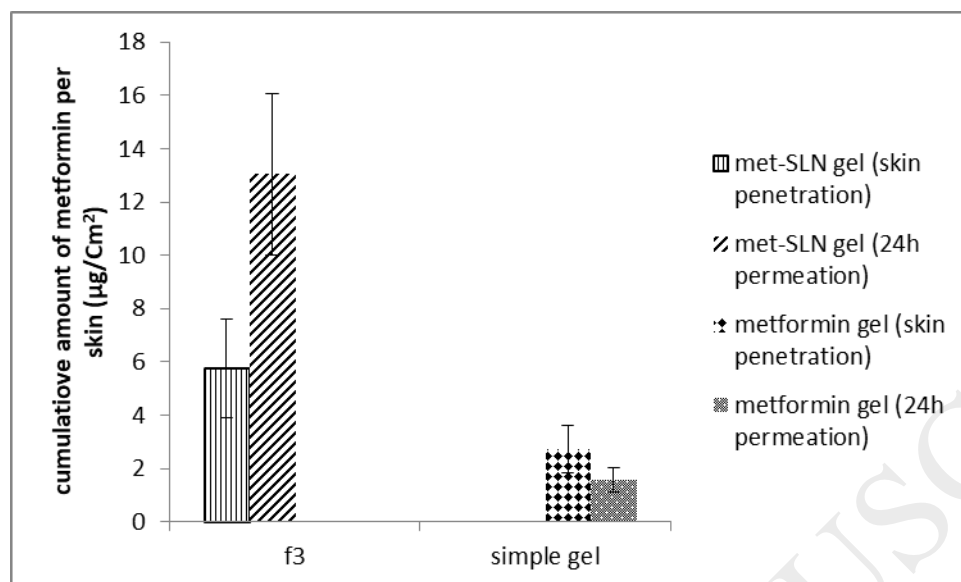


Figure 6. Amount of Met penetrating to the skin layers (Dermal delivery) (error bars are standard deviation, sample represents Met-SLN and standard formulation is the Met solution gel containing the same concentration of Met; t-test was carried out between the sample and standard. The difference was significant $p < 0.05$; $n = 3$).

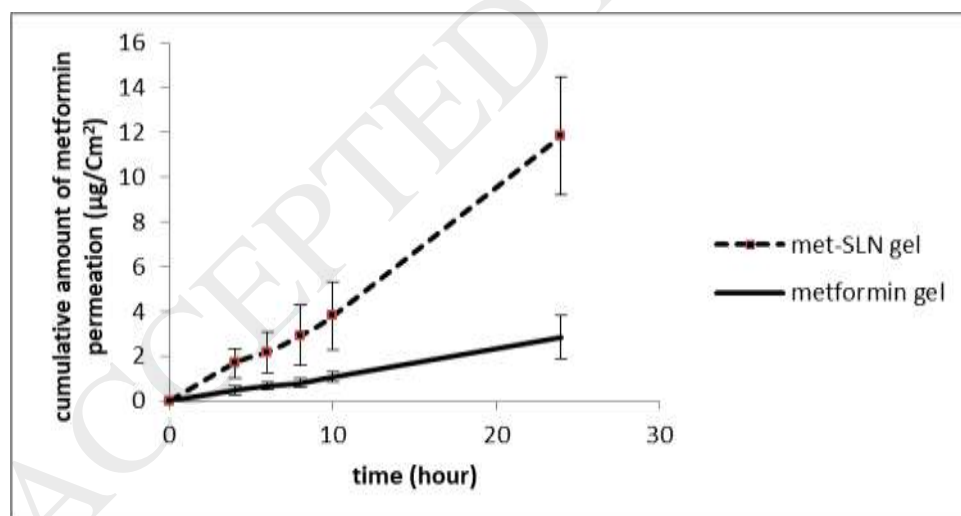


Figure 7. Cumulative amount of Met permeated across rat skin (data is mean and standard deviation of three determinations, $n = 3$; ANOVA test followed by Tukey's test showed that the effect of time and formulation on Met permeation was significant $p < 0.05$). Sample represents Met-SLN gel and standard is Met solution gel containing the same concentration of Met.